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### Method

1 The invention relates to methods for phenotyping by determining protein activity *in vivo* using a probe compound and enhancing the nuclear polarisation of NMR active  
5 nuclei present in the probe compound (hereinafter termed "hyperpolarisation") prior to NMR analysis.

10 The ability of an organism to absorb a drug, translocate it, break it down (metabolise it) and finally remove it from the organism itself is crucial to how well a drug will operate in a particular organism or individual. For clinical trialing of a new drug as well as for the therapeutic efficacy of a drug it is important to gain better understanding of the performance of a drug in a human individual or a human population. Thus, an attempt was made to classify populations into groups of individuals with similar biological characteristics and behaviours. This attempt has  
15 become known as phenotyping. In the context of the present invention, a phenotype is defined in one of the three distinct ways: i) the totality of the observable functional and structural characteristics of an organism as determined by interaction of the genotype of the organism with the environment in which it exists, ii) any particular characteristic or set of characteristics of a organism so determined and iii) a group of  
20 organisms exhibiting the same set of such characteristics.

Clinical trialing of a new drug in the human population is an expensive and protracted process. Late failure of a putative drug has a significant impact on the profitability of the developer, while withdrawal of a drug after its launch on the open  
25 market has an even greater impact on the valuation and reputation of a pharmaceutical company. Phenotyping of a clinical trial group is therefore potentially very valuable in understanding how individuals respond beneficially or adversely to a new drug. Using volunteer patients for clinical trials of defined phenotypes facilitates the design of clinical phase I and II protocols and the  
30 interpretation of clinical data and potential adverse drug reactions during the trial can be reduced.

Therapeutic efficacy of a drug is dependent on if and how individuals respond to the administered drug. On the basis of the extent to which a therapeutic drug is  
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metabolised, individuals might be characterised as being extensive, normal or poor metabolisers of a therapeutic drug.

5 In normal metabolisers, steady-state drug levels are within the expected therapeutic range and toxic effects are absent whilst in extensive metabolisers, steady-state drug levels are sub-therapeutic which can lead to no drug effect at all. In poor metabolisers, steady-state drug levels are larger than expected and these individuals are thus susceptible to undesired toxicity or other adverse effects of the drug. Thus, phenotyping of an individual receiving therapeutic drug treatment is valuable in  
10 understanding how individuals respond to certain drugs and drug doses and it is potentially helpful in determining adequate drugs and drug doses in order to achieve optimal therapeutic results.

15 Metabolism and transport of drug molecules in the human or non-human animate body are governed by certain proteins, e.g. enzymes or transporter proteins. The determination of the activity of said proteins can be used to phenotype individuals. Cytochrome P 450 (CYP450) plays a key role in the metabolism of drugs. The members of the CYP450 superfamily of oxidases show a common catalytic mechanism but individual isoenzymes have divergent substrate specificity. In order  
20 to assess the multiplicity of the CYP450 isoenzymes it is not possible to study metabolism with a single probe compound but several probe compounds which act as substrates for different CYP450 isoenzymes have to be used.

25 R. J. Scott et al., Rapid Commun. Mass Spectrom. 13, 1999, 2305-2319 describe the use of a "cocktail" of multiple probe drugs for studying the *in vitro* metabolism of said cocktail in human urine or plasma samples upon addition of the enzyme  $\beta$ -glucuronidase. After reaction, the samples were worked up by solid phase extraction and analysed by liquid chromatography and mass spectrometry (LC/MS/MS). The disadvantage of this method is that work-up of the samples is time consuming.  
30 Moreover, due to reduced recovery of the probe drugs and their metabolites after solid phase extraction it might be difficult to detect small amounts of metabolites. Another disadvantage is that any small change in the method itself requires a careful validation.

In WO-A-00/35900 several probe drugs comprising phenolic dyes are used for *in vitro* screening assays as optical probes or sensors of the activity of CYP450 isoenzymes. The disadvantage with this method is that the addition of dye may influence the metabolic breakdown. Moreover, optical measurements may not be sufficiently specific due to, e.g. dye leakage, dye compartmentalisation or quenching of signals. Although the method provides an indication of potential drug-drug interactions, it is far removed from the real *in vivo* situation. Thus, the method can only be employed as an initial screening method.

10 K. Akira et al., Drug Metab. Dispos 29, 2001, 903-907 describe the use of <sup>13</sup>C-labelled antipyrine as an *in vivo* probe to evaluate some CYP450 isoenzymes using <sup>13</sup>C-NMR spectroscopy. Due to the reduced sensitivity of conventional <sup>13</sup>C-NMR spectroscopy, the probe drug has to be administered in large amounts leading to potential risk of adverse drug effects in the patients.

15

WO-A-01/96895 describes a method for obtaining information regarding the fate of a test compound in a biological system by enhancing the nuclear polarisation of an NMR active nuclei present in the test compound (hyperpolarisation) prior to NMR analysis.

20

Thus, there was a need for a fast and simple method for phenotyping of human individuals.

The present invention provides a method for phenotyping of a human individual comprising determining *in vivo* protein activity and thereby obtaining a characteristic of said human individual, the determination comprising

- a) selecting at least one probe compound containing at least one NMR active nuclei
- b) administering said probe compound to said human individual
- 30 c) collecting samples from said human individual
- d) hyperpolarising the NMR active nuclei of said samples and
- e) analysing said samples by NMR spectroscopy.

In the context of the present invention, "proteins" means all proteins whose activity can be influenced by a probe compound acting e.g. as a substrate, inducer or inhibitor of said protein. Preferred proteins are enzymes and transporter proteins, e.g. NADPH quinone oxireductases, preferably CYP450, N-acetyltransferase, glutathione transferase, serotonin transport protein, and p-glycoprotein.

Protein activity can be determined in a number of ways, e.g. by measuring the rate of disappearance of the probe compound from plasma with time. As this has been a difficult and time consuming task, measuring the ratio of the probe compound to their metabolites at one or more selected time points is preferred.

In step a) of the method according to the invention, at least one probe compound is selected which contain at least one NMR active nuclei.

Preferably, the probe compound and its metabolites show a well-dispersed NMR spectrum in order to clearly distinguish between the single probe compounds and their metabolites. Furthermore, the probe compound should be safe and available. It is further preferred that the probe compound is selected so that it is possible to analyse the probe compound and its metabolites in different samples collected from the human individual, particularly in different biofluids.

The selection according to step a) is dependent on which protein activity is going to be determined. If the activity of an enzyme family comprising several isoenzymes is to be determined either several probe compounds are selected and the method according to the invention is repeatedly carried out using a single probe compound or several probe compounds are selected and the method according to the invention is carried out using said several probe compounds. Thus, suitably at least 3 probe compounds are selected, most suitably at least 4 and preferably at least 7 probe compounds.

30

If the enzyme family to be addressed in the method according to the invention is CYP450, a number of possible probe compounds for different isoenzymes are known (see for example R. J. Scott et al., Rapid Commun. Mass Spectrom. 13, 1999,

2305-2319 or R.F. Frye et al., Clin. Pharmacol. Ther. 62, 1997, 365). Said probe compounds are preferably selected according to the above-mentioned aspects.

Suitably, the probe compounds are substrates for CYP450, preferably substrates for CYP450 isoenzymes selected from the group consisting of CYP1A2, CYP2A6, CYP2B6, CYP2C8/9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4.

Preferably, the probe compounds are substrates for CYP450 selected from the group consisting of Phenacetin, Coumarin, Tolbutamide, Mephenytoin, S-Mephenytoin, Bufuralol, Chlorzoxazone, Midazolam, Caffeine, Dapsone, Diclofenac, Debrisoquine, Bupropion, Antipyrine and Dexomethorphan.

The probe compound according to the invention contains at least one NMR active nuclei, i.e. a nuclei with non-zero nuclear spin. Preferred nuclei are  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^{31}\text{P}$ ,  $^{19}\text{F}$ , and/or  $^1\text{H}$ . Isotopically enriched probe compounds can be employed. If non-enriched probe compounds are employed, probe compounds containing nuclear species occurring at high natural abundance such as  $^{31}\text{P}$ ,  $^{19}\text{F}$ , and/or  $^1\text{H}$  are employed. However, isotopically enriched probe compounds, preferably enriched with non-radioactive isotopes, are preferably used in the method according to the invention as the isotopic enrichment has substantially no effect on the therapeutic efficacy of the probe compound and the NMR detection is strongly facilitated.

The enrichment may include either selective enrichments of one or more sites within the probe compound molecule or uniform enrichment of all sites. Preferably, the probe compounds are isotopically enriched in only one position of the molecule. Enrichment can be achieved by chemical synthesis or biological labelling. Suitably, a probe compound for use in the method according to the invention is an organic compound isotopically enriched in only one position of the molecule with an enrichment of at least 10%, most suitably at least 25%, preferably at least 75%, most preferably at least 90%, ideally approaching 100%.

In a preferred embodiment of the present invention, the probe compound is enriched with  $^{13}\text{C}$  and/or  $^{15}\text{N}$ , preferably with  $^{13}\text{C}$  or  $^{15}\text{N}$ , particularly preferred with  $^{13}\text{C}$  as for higher sensitivity and a broader choice of labelling. In a further preferred

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embodiment, all probe compounds are enriched with the same NMR active nuclei. Thus, it is possible to collect information in a single NMR analysis.

5 The optimal position for isotopic enrichment in the probe compound is dependent on the relaxation time of the NMR active nuclei. Preferably, probe compounds are isotopically enriched in positions with long T1 relaxation time. Further, the probe compounds are preferably isotopically enriched at positions in the molecule where upon metabolism structural changes take place. This leads to greater chemical shift differences between the probe compound and its metabolites which lead to better  
10 dispersed NMR spectra. Labelling in two or more positions may facilitate the interpretation of complex NMR spectra.

In step b) of the method of the invention, the probe compound is administered to the human individual.

15

The probe compound is preferably dissolved or dispersed in a solvent or solvent mixture which can be used in connection with administration to a human individual. The usual mixing techniques such as stirring, bubbling, agitation, vortexing or sonification can be applied. In another embodiment, a solid probe compound is  
20 administered.

If more than one probe compound is administered, the probe compounds can either be administered sequentially or as a mixture of probe compounds.

25 If the probe compounds are administered in a mixture, probe compounds can be mixed and subsequently dissolved or dispersed in a solvent or a solvent mixture which can then be directly used for administration or which can be further treated before the administration. Alternatively, each probe compound or some of the probe compounds are dissolved or dispersed in a solvent or a solvent mixture first and then  
30 a mixture of the dissolved / dispersed probe compounds is prepared. In order to achieve proper mixing the usual mixing techniques such as stirring, bubbling, agitation, vortexing or sonification. In another embodiment, mixtures of solid probe compounds are provided.



For administration, the probe compound is preferably formulated in conventional pharmaceutical or veterinary administration forms. If the probe compound is administered in solution than it may be in the form of a suspension, dispersion, slurry etc., for example in an aqueous vehicle such as water. If the probe compound  
5 is administered in solid form , than it may be in the form of tablets or powder.

For administration, the probe compound may further contain pharmaceutically acceptable diluents and excipients and formulation aids e.g. stabilisers, antioxidants, osmolality adjusting agents, buffers or pH-adjusting agents. For injection, a sterile  
10 solution or suspension of the probe compound is most preferred. For parental administration, a carrier medium which is preferably isotonic or somewhat hypertonic, is preferred.

The probe compound is preferably administered into the vasculature or directly into  
15 an organ or muscle tissue as well as subdermally or subcutaneously. In another preferred embodiment, the probe compound is administered via non-parental route such as transdermal, nasal, sub-lingual or into an external body cavity e.g. orally in the gastro-intestinal tract.

20 The dosage for administration is preferably therapeutic or sub-therapeutic, particularly preferably sub-therapeutic. Due to the sensitivity of the method according to the invention, sub-therapeutic administration is possible which strongly minimises the risk of adverse effects of the probe compounds.

25 In step c) of the method of the invention, samples from said human individual are collected. Samples may be taken once, at time intervals or continuously (dynamic studies)

Samples that may be collected include tissue or cell samples, faeces, biofluids  
30 including but not limited to blood, blood plasma, lymph, urine, semen, breast milk, cerebro-spinal fluid, sweat, lachrymal or parotid secretions or lavage. Preferably, samples collected are biofluids, particularly preferably blood, blood plasma or urine.

If the method according to the invention is used for determining the *in vivo* activity of CYP450 isoenzymes, collected samples are preferably blood, blood plasma and urine.

- 5 The collected samples may be further processed, e.g. in order to separate cells from liquids. Thus, blood may be treated in order to obtain blood plasma. The samples may be purified prior to hyperpolarisation and/or analysis but this is not always necessary. An important advantage of the method according to the invention is that analysis can be carried out directly on the crude sample without the need for  
10 fractionation, purification or concentration steps.

- If the protein activity is determined by calculating the rate of disappearance of the probe compound, a reference standard may conveniently be included in the sample before hyperpolarisation. Inclusion of said standard allows the determination of the  
15 concentration of the probe compounds and their metabolites. Preferably, one standard is added. Suitable standards are simple molecules comprising signals which do not interfere with the signals from the probe compounds and their metabolites. Preferred standards do comprise only one signal. Conveniently, a chemical shift reference is added to the sample before hyperpolarisation.

- 20 In step d) of the method of the invention, the NMR active nuclei of said samples are hyperpolarised.

- There are several ways for hyperpolarising NMR active nuclei, preferred ways are  
25 polarisation transfer from a noble gas, "brute force", DNP and spin refrigeration, all explained below.

- A preferred way for hyperpolarising the NMR active nuclei containing probe compounds according to the invention is the polarisation transfer from a  
30 hyperpolarised noble gas. Noble gases having non-zero nuclear spin can be hyperpolarised, i.e. have their polarisation enhanced over the equilibrium polarisation, e.g. by the use of circularly polarised light. A hyperpolarised noble gas, preferably  $^3\text{He}$  or  $^{129}\text{Xe}$ , or a mixture of such gases, may be used according to the present invention to effect hyperpolarisation of the NMR active nuclei present in the  
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probe and/or test compounds. The hyperpolarisation may also be achieved by using an artificially enriched hyperpolarised noble gas, preferably  $^3\text{He}$  or  $^{129}\text{Xe}$ . The hyperpolarised gas may be in the gas phase, it may be dissolved in a liquid, or the hyperpolarised gas itself may serve as a solvent. Alternatively, the gas may be condensed onto a cooled solid surface and used in this form, or allowed to sublime. Either of these methods may allow the necessary intimate mixing of the hyperpolarised gas with the target to occur. In some cases, liposomes or microbubbles may encapsulate the hyperpolarised noble gas.

Another preferred way for hyperpolarising the NMR active nuclei containing probe compounds according to the invention is that polarisation is imparted to said NMR active nuclei by thermodynamic equilibration at a very low temperature and high field. Hyperpolarisation compared to the operating field and temperature of the NMR spectrometer is effected by use of a very high field and very low temperature (brute force). The magnetic field strength used should be as high as possible, suitably higher than 1 T, preferably higher than 5 T, more preferably 15 T or more and especially preferably 20 T or more. The temperature should be very low, e.g. 4.2 K or less, preferably 1.5 K or less, more preferably 1.0 K or less, especially preferably 100 mK or less.

Another preferred way for hyperpolarising the NMR active nuclei containing probe compounds according to the invention is the DNP (dynamic nuclear polarisation) method effected by a DNP agent. DNP mechanisms include the Overhauser effect, the so-called solid effect and the thermal mixing effect. Most known paramagnetic compounds may be used as DNP agents, e.g. transition metals such as chromium (V) ions, magnesium (II) ions, organic free radicals such as nitroxide radicals and trityl radicals (WO-A-98/58272) or other particles having associated free electrons. Preferably, radicals with low relaxivity are used as DNP agents. Where the DNP agent is a paramagnetic free radical, the radical may be conveniently prepared *in situ* from a stable radical precursor by a radical-generating step shortly before the polarisation, or alternatively by the use of ionising radiation. During the DNP process, energy, normally in the form of microwave radiation, is provided, which will initially excite the paramagnetic species. Upon decay to the ground state, there is a transfer of polarisation to the NMR active nuclei of the target material. The

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method may utilise a moderate or high magnetic field and a very low temperature, e.g. by carrying out the DNP process in liquid helium and a magnetic field of about 1 T or above. Alternatively, a moderate magnetic field and any temperature at which sufficient NMR enhancement is achieved in order to enable the desired studies to be carried out may be employed. The method may be carried out by using a first magnet for providing the polarising magnetic field and a second magnet for providing the primary field for MR spectroscopy.

Another preferred way for hyperpolarising the NMR active nuclei containing probe and/or test compounds according to the invention is the spin refrigeration method. This method covers spin polarisation of a solid compound or system by spin refrigeration polarisation. The system is doped with or intimately mixed with suitable paramagnetic materials such as  $\text{Ni}^{2+}$ , lanthanide or actinide ions in crystal form with a symmetry axis of order three or more. The instrumentation is simpler than required for DNP with no need for a uniform magnetic field since no resonance excitation field is applied. The process is carried out by physically rotating the sample around an axis perpendicular to the direction of the magnetic field. The prerequisite for this method is that the paramagnetic species has a highly anisotropic g-factor. As a result of the sample rotation, the electron paramagnetic resonance will be brought in contact with the nuclear spins, leading to a decrease in the nuclear spin temperature. Sample rotation is carried out until the nuclear spin polarisation has reached a new equilibrium.

Some of the hyperpolarisation techniques described above, e.g. DNP, brute force or spin refrigeration transfer, are only effective when transferring polarisation to the solid state. If the sample is not solid, it may conventionally be frozen in an appropriate solvent or solvent mixture prior to hyperpolarisation by one of the methods that needs to be carried out in the solid state. Solvent mixtures have been found to be particularly suitable, especially if the mixture forms an amorphous glass, preferably by use of glycerol. Such a matrix of amorphous glass is preferably employed in DNP hyperpolarisation to ensure homogenous intimate mixing of radical and target in the solid.

The degree of hyperpolarisation of the NMR active nuclei according to the invention can be measured by its enhancement factor compared to thermal equilibrium at spectrometer field and temperature. Suitably the enhancement factor is at least 10, preferably at least 50 and more preferably at least 100. However, methods according to the invention where even smaller enhancements are achieved may still be performed usefully due to the shorter time needed for the total measurement compared with methods described in the prior art. If the enhancement is reproducible and the hyperpolarisation/NMR analysis can be repeated, the signal to noise ratio of a NMR signal can be improved. In such a case, the minimum NMR enhancement factor required depends on the hyperpolarisation technique and the concentration of the probe/test compound and their metabolites. The enhancement has to be large enough so that the NMR signal from the probe/test compound and their metabolites can be detected. In this context, it is clear that an enhancement of 10 or less than 10 that is achievable in a multi-shot experiment may be very useful due to the time saved in data acquisition compared with conventional NMR.

In step e) of the method of the invention the samples from step d) are analysed by NMR spectroscopy. The analysis may be carried out by continuous monitoring or as a single discrete measurement or as a series of discrete measurements that may be carried out at suitable intervals over time. Thus, it is possible to identify many and preferably all changes in metabolism and appearance of individual metabolites of the probe compounds.

The hyperpolarised sample may as well be further diluted or mixed with suitable solvents or solvent mixtures, for NMR spectroscopy, depending on which kind of NMR analysis, e.g. liquid or solid phase NMR spectroscopy has to be applied.

After hyperpolarisation, it is desirable to preserve as much as possible of the polarisation prior to NMR analysis. Some of the hyperpolarisation techniques described above, e.g. by DNP, brute force, spin refrigeration transfer, are only effective when transferring polarisation to the solid state. However, it is often desired to investigate the NMR spectrum of a sample in the liquid state, in order to improve spectral resolution and sensitivity. Alternatively, line-narrowing techniques like

Magic Angle Spinning (MAS) can be employed to increase spectral resolution of NMR in the solid state and enable low temperature NMR analysis.

5 If a liquid state NMR technique is to be employed, once the sample has been hyperpolarised, it can be rapidly removed from the polarisation chamber and then dissolved in a suitable solvent. It is advantageous to use solvents which do not interfere with the spectra produced in the analysis step or solvents which keep a stable chemical environment and prolong the T1 relaxation time. Deuterated solvents such as D<sub>2</sub>O or mixtures of methanol and acid, preferably with an excess of  
10 methanol, are particularly suitable. Stirring, bubbling, sonification or other known techniques can be used to improve the speed of dissolution. Suitably, the temperature and the pH of the solution are maintained to allow optimal dissolution and a long nuclear relaxation time.

15 Preferably, the sample or a solution thereof is kept in a holding field throughout the period between polarisation and analysis in order to prevent relaxation. A holding field provides a higher field than the Earth's magnetic field and suitably higher than 10 mT. It is suitably uniform in the region of the sample and the optimal conditions will depend on the nature of the sample.

20

The sample or a solution thereof is subsequently transferred for examination by standard solution phase NMR analysis. The transfer process is manually or automated, preferably automated. Alternatively, the hyperpolarisation step and optional subsequent dissolution steps are suitably integrated into a single automated  
25 unit. In an additional suitable embodiment, hyperpolarisation and optional dissolution steps are automated and NMR detection hardware is also housed within the same single fully integrated unit.

Alternatively, where a solid state NMR technique is to be used, the solid state  
30 sample may be hyperpolarised, e.g. by DNP, brute force, spin refrigeration transfer or any other method that will work in the solid state at low temperature. Subsequently, the hyperpolarised sample will be moved into a solid state MAS NMR probe. The movement is suitably rapid and is preferably carried out via lifting or ejection. The sample in the NMR probe will then be spun so that high resolution

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solid state NMR spectroscopy can be carried out. The entire process can be automated and will preferably be carried out in an integrated unit.

5 The method of the invention can be used for phenotyping of human individuals, e.g. for phenotyping of a clinical trial group or for phenotyping of individuals prior to therapeutic drug treatment.

10 If the method according to the invention is used for phenotyping of a clinical trial group, protein activity according to the invention is determined in the volunteer patients. According to the characteristic obtained for each volunteer patient, said volunteer patients can be classified into groups of volunteer patients exhibiting similar or same characteristic. Thus, it is possible to start a clinical trial with volunteer patients showing a specific phenotype.

15 If the method according to the invention is used for phenotyping of a human individual prior to therapeutic drug treatment, the characteristic of said human individual obtained by the method of the invention can be compared characteristics of other human individuals already grouped according to their characteristics. Thus, it is possible to classify said human individual into a group.

20 Another aspect of the present invention is a method for phenotyping comprising determining *in vivo* protein activity and thereby obtaining a characteristic of said human individual, the determination comprising

- 25 a) collecting samples from said human individual preadministered with at least one probe compounds containing at least one NMR active nuclei,  
b) hyperpolarising the NMR active nuclei of said samples and  
c) analysing said samples by NMR spectroscopy.

30

## Examples

### 5 1) Selection of probe compounds

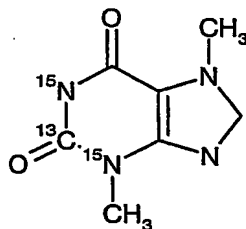
The activity of the CPY450 isoenzymes CYP1A2, CYP2D6 and CYP2E1 was determined using the following probe compounds :

- 10 • Caffeine as a substrate for CYP1A2, caffeine is primarily metabolised to paraxanthine.
- Debrisoquine as a substrate for CYP2D6, debrisoquine is primarily metabolised to 4-hydroxy-debrisoquine.
- Chlorzoxazone as a substrate for CYP2E1, chlorzoxazone is primarily metabolised to 6-hydroxy-chlorzoxazone

15

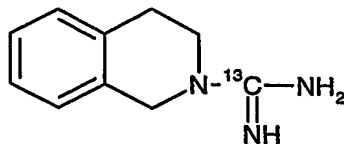
The compounds were isotopically labelled at the following positions:

Caffeine:



20

Debrisoquine:

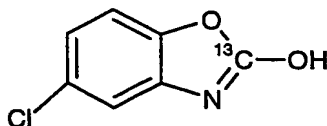


25



## Chlorzoxazone

5



2.5 mg of each of the probe compounds were dissolved in 1 ml 10 mM sodiumphosphate buffer, pH 7.3, 0,9% NaCl.

10

## 2) Administration of probe compounds and collection of samples

SPD rats 1-4 were injected interperitonal (i.p) with either the individual probe compound solutions a mixture thereof. Urine samples were collected immediately before administration, and then at 3 and 6 h after administration. The volume of urine collected for each period was in the range of 1 to 10 ml for each of the subjects. Blood samples were collected immediately before administration and then at 3 hours after administration. After collection, blood samples were spun down at 2000 rpm for 10 min. and the plasma was collected. Both plasma and urine samples were frozen at  $-20^{\circ}\text{C}$ . 1 ml of each urine sample was freeze dried overnight.

20

## 3) Hyperpolarisation of the collected samples and NMR analysis

The freeze dried urine samples were dissolved in 100  $\mu\text{l}$  water. 40  $\mu\text{l}$  of said solutions were mixed with glycerol in a 50:50 w/w ratio and quickly frozen in liquid nitrogen. The frozen samples were placed within the DNP magnet and hyperpolarised overnight. Subsequently, the samples were dissolved in a mixture of methanol and acetic acid (100:1) and  $^{13}\text{C}$  NMR spectra were acquired. A similar procedure as described with urine was performed with the blood plasma samples.

25

30

#### 4) Results

A large number of signals were expected for the probe compounds and their multitudes of metabolites. In addition the bio-fluid matrix signals and the solvent signals were also expected to be present in the spectra.

- 5 For caffeine at least 8 metabolites in addition to caffeine itself were present in the spectrum. Major caffeine metabolites were expected to be 1,3-dimethyl uric acid; 1, 3, 7-trimethyl uric acid and paraxanthine. Further minor caffeine metabolites were expected to be 1-xanthine, 1, 3-xanthine; 3, 7-xanthine; 1, 3, 7-DAU; 3, 7-uric acid; 1, 7-uric acid and 1-uric acid.
- 10 For debrisoquine, several metabolites including 4-hydroxy debrisoquine were present in addition to debrisoquine itself. In the urine sample collected after 3 h, only debrisoquine and 4-hydroxy debrisoquine were present. Additionally, two unknown signals were present which were possibly urine background signals.
- 15 The signals from the probe compounds and their primary metabolites were the most intense signals in the most suitable bio-fluid and at the most suitable time. From these signals it is possible to determine enzyme activity by calculating a metabolic ratio. The metabolic ratio is calculated as the percentage of dose excreted in urine as unchanged probe compound divided by the percentage excreted as the metabolite
- 20 formed via the pathway catalysed by the particular enzyme responsible for the primary metabolite. Thus, said metabolic ratio is a measure of the individual CYP450 isoenzyme activity.



## Claims

1. Method for phenotyping comprising determining *in vivo* protein activity and thereby obtaining a characteristic of said human individual, the determination comprising
  - a) selecting at least one probe compound containing at least one NMR active nuclei
  - b) administering said probe compound to said human individual
  - c) collecting samples from said human individual
  - d) hyperpolarising the NMR active nuclei of said samples
  - e) analysing said samples by NMR spectroscopy
2. Method according to claim 1 wherein the method is used to determine protein activity of several proteins or isoenzymes thus obtaining a set of characteristics of said human individual.
3. Method according to claims 1 and 2 wherein characteristics of several human individuals are obtained .
4. Method according to claim 3 wherein said human individuals who exhibit the same or similar characteristics are grouped.
5. Method according to claim 4 for phenotyping of a clinical trial group
6. Method according to claims 1 and 2 wherein said characteristic of said human individual is compared with characteristics of other human individuals obtained according to claim 4 and thereby classifying said human individual into a group.
7. Method according to claim 6 for phenotyping of a human individual prior to said human individual receives therapeutic drug treatment.

8. Method according to claim 1 to 7 wherein the probe compound is enriched with NMR active nuclei.
- 5 9. Method according to claims 1 to 8 wherein hyperpolarisation is carried out by means of polarisation transfer from a noble gas, brute force, dynamic nuclear polarisation (DNP) or spin refrigeration.
- 10 10. Method according to claims 1 to 9 wherein the probe compound is administered into the vasculature, an organ, tissue or via a non-parental route.
11. Method according to claims 1 to 10 wherein the collected samples are biofluids.
- 15 12. Method according to claim 1 to 11 wherein said probe compound is a substrates for Cytochrome P 450 (CYP450)
- 20 13. Method according to claim 12 wherein said probe compound is a substrates for CYP 450 isoenzymes selected from the group consisting of CYP1A2, CYP2A6, CYP2C8/9, CYP2C19, CYP2D6, CYP2E1 and CYP3A.



### Abstract

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The invention relates to methods for phenotyping by determining protein activity *in vivo* using a probe compound and enhancing the nuclear polarisation of NMR active nuclei present in the probe compound (hereinafter termed "hyperpolarisation") prior to NMR analysis.

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